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## Mycorrhizal Colonization and Nutrient Uptake of Dry Bean in Manure and Compost Manure Treated Subsoil and Untreated Topsoil and Subsoil

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### ABSTRACT

Eroded or leveled Portneuf silt loam soils (coarse-silty mixed mesic Durixerollic Calciorthid) have been restored to topsoil productivity levels by manure application, but not by other organic sources such as cheese whey. In dry bean (*Phaseolus vulgaris* L. cv. Viva), only soil organic matter and Zn concentration of leaf tissue correlated with improved yields. Manure application could potentially increase or decrease mycorrhizal colonization depending on which factors dominate. Manured and unmanured soils from a long-term field experiment were sampled and mycorrhizal spores were quantified, but there was no significant manure treatment effect on spore numbers. A greenhouse study was conducted to see if manure or composted manure freshly applied to subsoils would facilitate mycorrhizal colonization in dry bean roots compared to untreated topsoil or conventionally fertilized subsoil. Low level colonization (< 5%) was observed 21 days after planting

and that increased to 58% by 56 days after planting. Roots grown on subsoil treated with manure or composted manure showed higher percent colonization than roots from untreated subsoil, but roots on topsoil had highest colonization. This increase in colonization was statistically significant for the last two sampling dates. Topsoil promoted the greatest percent colonization in early bean growth and this was reflected in greater Zn uptake during early growth stages. By day 56, plants grown in manured subsoil absorbed Zn equal to topsoil and at higher levels than the subsoil control. However, this increase in Zn uptake was not seen in plants grown in compost manured subsoil. A decrease in root and shoot weight was observed in the composted manure treatment and this seemed to decrease mycorrhizal efficiency. Uptake of other nutrients was either not related or was negatively related to mycorrhizal infection. The higher percent colonization of roots by mycorrhizal fungi stimulated by manure could explain the field observations of higher bean yield and Zn contents in dry bean in manured than in untreated subsoils.

## INTRODUCTION

Land leveling and irrigation-induced topsoil erosion of silt loam soils in south-central Idaho have affected the yields on nearly 800,000 ha of subsoil. Because crop productivity on subsoil is negatively affected, Robbins et al. (1997) assessed the potential to restore subsoil productivity to the same level as in Portneuf silt loam topsoil using varying amendments and crop rotations. The rotations were established on treated subsoil and topsoil with untreated topsoil as the control and grown over a three-year period. Soil amendment treatments applied to subsoil plots were conventional fertilizer, dairy manure, and cottage cheese whey. During the fourth year, dry bean was grown on all treatments and rotations to evaluate rotation/amendment effects. Yield and nutrient composition of dry bean were assessed. Rotations did not affect yield and only the manure treatment brought productivity of subsoil up to that of topsoil. The only factors that correlated with these improved dry bean yields were soil organic matter and plant zinc (Zn) concentration. Several potential explanations for these observations exist including increased mycorrhizal colonization, increased solubilization of Zn by short-chained organic molecules, and/or improved bulk density and root development.

Mycorrhizae and plant roots form an important symbiotic relationship in most plant species. The plant provides the fungus with photosynthate and the fungus can provide improved nutrient and water uptake, salinity or drought tolerance, root disease resistance, and increased photosynthesis (Raman and Mahadevan, 1996; Sharma et al., 1994). Mycorrhizal extension of the plant root surface facilitates potential uptake and translocation of phosphorus (P), zinc (Zn), nitrogen (N), potassium (K), calcium (Ca), sulfur (S), copper (Cu), and molybdenum (Mo) (Raman and Mahadevan, 1996; Singer and Munns, 1987; Azcon-Aguilar and Barea, 1992).

Dry bean is susceptible to Zn deficiency (Shukla and Raj, 1987; Ambler and Brown, 1969) and physiological characteristics of species and/or cultivars are part of the reason these plants are susceptible (Jolley and Brown, 1991). Dry bean is known to develop mycorrhizal relationships (Vivekanandan and Fixen, 1991). Mycorrhizal colonization of dry bean roots can also alleviate Zn deficiencies and increase plant Zn uptake (Leggett and Westermann, 1986). In addition to direct benefit to the plant, arbuscular mycorrhizal fungi (AMF) can increase nutrient availability to benefit  $N_2$  fixation (Mosse, 1973; Pacovsky and Fuller, 1986; Azcon-Aguilar and Barea, 1992; Hwang et al., 1993).

The relationship between soil organic matter and mycorrhizae development is complex. Mycorrhizal hyphae have been shown to increase in length due to organic matter (St. John et al., 1983). Mycelium from roots infected with mycorrhizal fungi will often grow rapidly around decaying root material and other organic matter (Nicolson, 1959). Hepper and Warner (1983) showed that mycorrhizae grew saprophytically in soil and that organic matter stimulated this process. Mosse (1959) stated that additions of farmyard manures or compost resulted in a greater number of root AMF entry points than additions of artificial fertilizers. This was attributed to manure affects on the root physiology which extends the period that the root is susceptible to AMF infection. An increased breakdown of organic matter by the mycorrhizal associations was noted. Ishac et al. (1986) found that organic matter amendments increased AMF colonization due to increased rooting intensity. Decreased mycorrhizal growth response has been linked to low soil organic matter (Kitt et al., 1988). Spores in low organic matter sandy soils were found primarily in particles of organic matter (Koske et al., 1975). In contrast, mycorrhizae tend to develop most extensively under low fertility conditions (Brechelt, 1989). Ellis et al. (1992) found that root colonization in sorghum and soybean was greatest when fertilizer was not added and lowest when manure was applied. Thus, manure application could potentially increase or decrease growth of mycorrhizae depending on which factors dominate.

The objectives of this study were (a) to determine if long-term field treatment of subsoil and topsoil with manure affected mycorrhizal fungi spore numbers compared to untreated topsoil and subsoil, (b) to determine if subsoil freshly treated with manure or composted manure would develop mycorrhizae similarly to untreated subsoil or topsoil, and (c) to relate these findings to the observed affects of manure on yield and Zn uptake observed by Robbins et al. (1997).

## MATERIALS AND METHODS

### Spore Counts

Soils from four field replications of four specific treatments [topsoil+manure added in 1994, topsoil+conventional fertilizer (-Zn), subsoil+manure added in 1991, subsoil+manure added in 1994, and subsoil+conventional fertilizer] were collected

in 14-L plastic buckets from long-term research plots at Kimberly, Idaho. The specific treatment history of these plots is described by Robbins et al. (1997). The treatments of manure added in 1994 were referred to as manure 95 because the first crop grown on these treatments was in 1995.

The soils from each treatment were sieved (5.6 mm) to remove small rocks and large root fragments, then thoroughly mixed. Approximately 1,000 g of soil were taken from each bucket and the spores were separated from 100 g of soil using a wet sieve, sucrose density gradient technique (Daniels and Skipper, 1982). Number of spores  $g^{-1}$  soil was determined using grided filter paper and a light microscope at magnification  $10\times$ . Samples from each field replication were analyzed in duplicate and spore counts of each of these duplicates were read twice.

### Greenhouse Study

Fresh soils (Portneuf silt loam) for the greenhouse experiment were obtained in October, 1996 from the USDA-ARS research plots in Kimberly, Idaho. Topsoil was collected at a depth of 0 to 30 cm and subsoils from 35 to 45 cm. Five Viva Pink dry beans  $pot^{-1}$  were planted, thinned to two plants  $pot^{-1}$  and grown in four soil amendment treatments each either inoculated or not inoculated with mycorrhizal fungi (*Glomus intraradices*). Treatments were topsoil, subsoil + manure, subsoil + manure compost and subsoil + fertilizer. Fertilizer applied was at an effective rate of  $45\text{ kg ha}^{-1}$  N as calcium nitrate,  $11\text{ kg ha}^{-1}$  Zn as zinc sulfate, and  $270\text{ kg ha}^{-1}$  P as concentrated superphosphate. Manure and composted manure were added at a rate of  $44\text{ magnesium (Mg) ha}^{-1}$  (dry weight). All soils were inoculated with *R. japonicum* prior to planting. Each treatment was repeated 12 times and arranged in a randomized complete block design. Two replications from each treatment were harvested 7, 14, 21, 28, 42, and 56 days after planting. Data for the 7- and 14-day harvests were not reported because of low colonization. Freshly harvested plant tops were rinsed lightly and plant roots were soaked in water and then rinsed thoroughly to remove all soil with minimal root disturbance. Roots and tops were separately dried at  $65^{\circ}\text{C}$  and dry weights recorded. Nitric acid/perchloric acid digestion and atomic absorption spectroscopy were used for determination of shoot concentrations of Zn, iron (Fe), Mn, Cu, Ca, Mg, and K (Johnson and Ulrich, 1959) for plants harvested 21, 28, 42, and 56 days after planting. Roots were rehydrated in tap water (Hetrick et al., 1989) and stained with aniline blue (Grace and Stribley, 1991) and percent mycorrhizal colonization determined using a point intersection method (Giovannetti and Mosse, 1980).

### Statistical Procedures

Spore count and greenhouse data were analyzed as randomized complete block designs using analysis of variance output from SAS (SAS Institute, 1989). Mean comparisons were made using a Student Newman Keuls procedure.

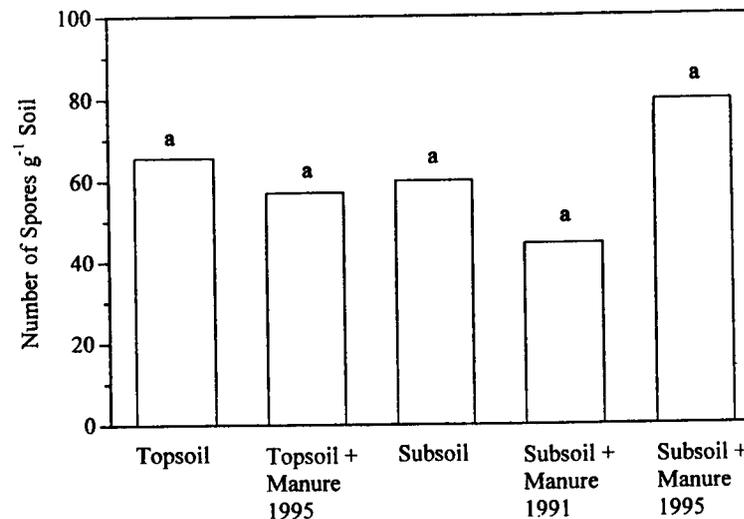


FIGURE 1. Number of mycorrhizal spores  $g^{-1}$  of soil under five field soil amendment treatments. Columns with the same letter are not significantly different at the 0.05 level, Student Newman Keuls.

## RESULTS AND DISCUSSION

### Spore Counts

One assessment of whether the correlation between organic matter and Zn uptake in dry bean was related to mycorrhizae would be spore counts made on soils previously treated in the field. Theoretically, there could be more spores in the soils with higher mycorrhizal colonization rates than for soils with low colonization rates. Consequently, one would project highest spore numbers on manured treatments and topsoil, but there were no significant differences in spore numbers between treatments (Figure 1). However, spore populations do not always correlate positively with colonization rates in soils (Smith and Read, 1997). Also, two additional crops had been grown on these soils since the observed differences in yield and Zn uptake of dry bean, and the manure effects on spore numbers may have diminished in these two years. However, organic carbon in these manured soils are still much higher ( $0.94\text{ mg kg}^{-1}$ ,  $1.26\text{ mg kg}^{-1}$ , and  $1.00\text{ mg kg}^{-1}$ , respectively, for subsoil+manure added in 1994, topsoil+manure added in 1994, and subsoil+manure added in 1991) than the untreated subsoil ( $0.47\text{ mg kg}^{-1}$ ) even in 1998 (three years later).

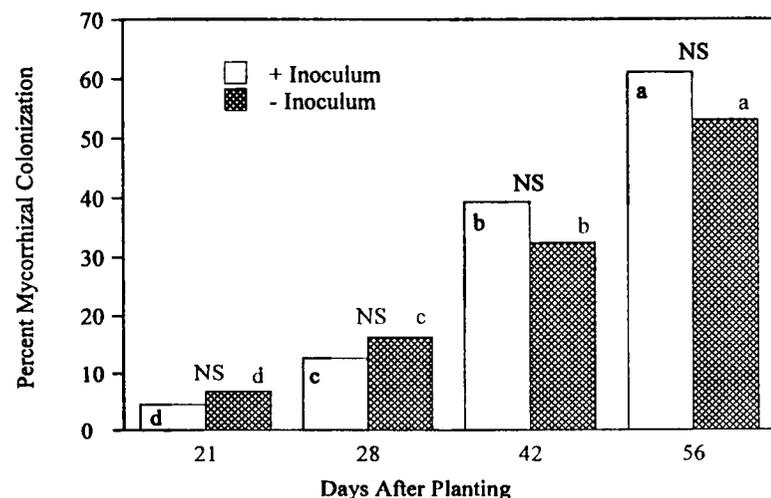


FIGURE 2. Percent mycorrhizal colonization of dry bean roots over time both with (+) and without (-) inoculum (*Glomus intraradices*). NS is not significant at 0.05 level comparing + and - inoculation, ANOVA. Columns for either plus or minus inoculum with the same letter are not significantly different from each other at the 0.05 level, Student Newman Keuls. Values for a given day are the average of all treatments.

### Greenhouse Study

No significant differences in AMF colonization were observed between inoculated and non-inoculated treatments (Figure 2). Since the freshly collected subsoil was taken from a depth of 30-60 cm and was known to be low in spores, these were unexpected results. However, the subsoil did contain pieces of alfalfa roots from the previous crop and even though the large root fragments were removed during sieving, small roots and hyphae may have remained and these could have acted as an inoculum source. Subsequent data reported are averaged over inoculum treatments.

Colonization percentages for days 7 and 14 after planting were negligible and are not reported. However, mean percent colonization significantly increased at each harvest date thereafter from 5 to 58% from 21 through 56 days after planting (Figure 2).

There was no harvest by treatment interaction for percent colonization. Dry bean roots in topsoil were colonized at a higher level than any other treatment. However, dry bean root mycorrhizal colonization was significantly impacted by

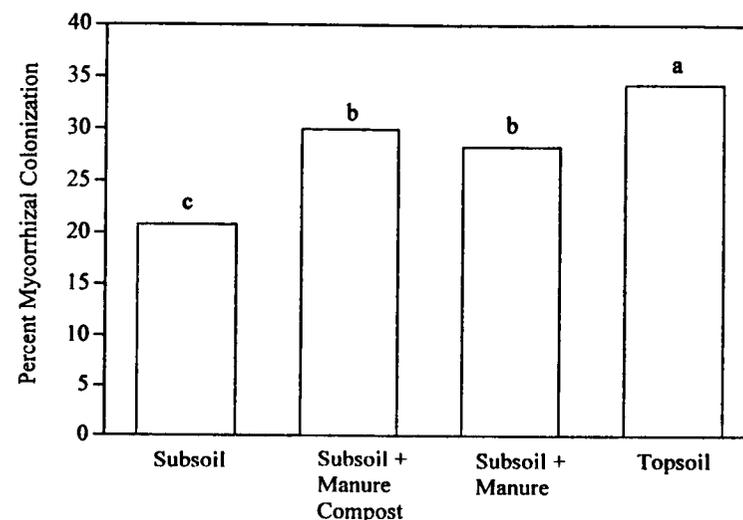


FIGURE 3. Percent mycorrhizal colonization (mean of all harvests and inoculation treatments) of dry bean roots under four soil treatments. Columns with the same letter are not significantly different at the 0.05 level, Student Newman Keuls.

manure and composted manure treatments compared to subsoil (Figure 3). These results corroborate results of others who attributed increased colonization in manured soils to increased fungal entry points (Mosse, 1959) or observed increased rooting intensity due to organic matter additions (Ishac et al., 1986). The effects of manure and composted manure on colonization were apparent as early as 21 days after planting, but they became statistically significant 42 and 56 days after planting (data not presented). Thus, manure additions to subsoil did increase the colonization of dry bean roots compared to subsoil in greenhouse pots; such an increase in the field would explain the increased uptake of Zn by dry bean on manured soils observed by Robbins et al. (1997).

Although there was a trend for manured subsoil treatment to increase Zn uptake in dry bean compared to the untreated subsoil as early as 42 days after planting, it was highly significant only by day 56 (Figure 4). Dry bean absorbed more Zn in the topsoil treatment when compared to the three subsoil treatments on days 21, 28, and 42 after planting. The improvement in Zn uptake in topsoil correlated with high mycorrhizal colonization (Figures 3 and 4). Improved Zn uptake in dry bean for the manured subsoil treatment corresponded with its increase in mycorrhizal colonization compared to the conventional subsoil treatment (Figures 3 and 4).

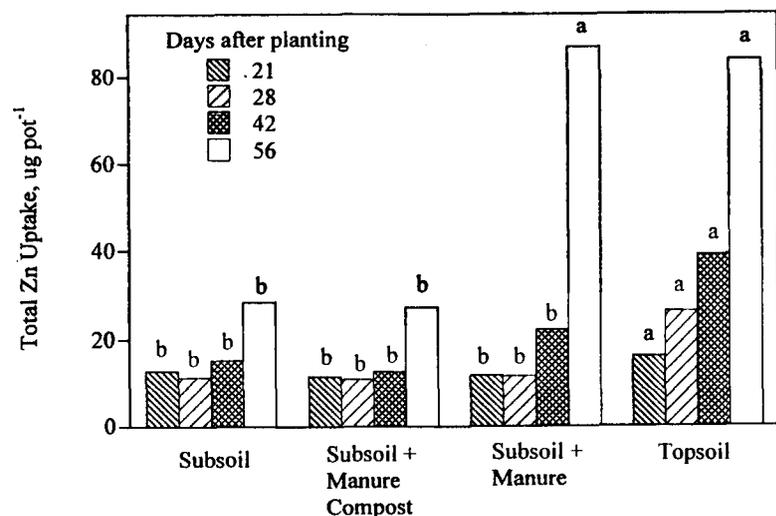


FIGURE 4. Total Zn uptake in dry bean under four treatments 21, 28, 42, and 56 days after planting. Columns with the same letter for a give day after planting are not significantly different at the 0.05 level, Student Newman Keuls.

These results corroborate research showing increased Zn uptake by mycorrhizae (Sharma and Srivastava, 1991; Sharma et al., 1994; Lambert and Weidensaul, 1991; Burkert and Robson, 1994; Hamilton et al., 1993; Swaminathan and Verma, 1979; Frey and Ellis, 1997). Because there was not a corresponding increase in root weight with increased colonization (Table 1) in the manured subsoil and topsoil treatments compared to subsoil, differences in mineral uptake would not be related to increased roots, but to mycorrhizae. The only explanation for the low Zn uptake associated with composted manure is a reduction in shoot and root weights associated with composted manure at days 42 and 56 (Table 1). Perhaps composted manure adversely affected growth in dry bean which negatively affected the mycorrhizal efficiency.

Dry bean uptakes of other nutrients were also affected by amendment treatments, and there were some significant treatment by harvest interactions (Table 1). However, none of these effects corresponded positively with mycorrhizal colonization as did Zn (Table 1 and Figure 3). Potassium uptake was essentially not affected by treatment except for day 21 plants. Iron uptake was highly variable and difficult to interpret. Manganese (Mn), Ca, and Mg uptakes were essentially highest in conventional subsoil compared to manured or topsoil treatments which

TABLE 1. Selected mineral uptake and root and shoot weights of dry bean under treatment.

Days after planting	Soil treatment	ug/pot		mg/pot				Root weight g pot <sup>-1</sup>	Shoot weight g pot <sup>-1</sup>
		Cu	Mn	Fe	Mg	K	Ca		
14	Subsoil	9.5 a	55 a	79 a	5.4 a	17 a	16 a	0.58 a	0.65 a
	Subsoil Compost Manure	9.2 a	34 b	81 a	4.1 b	18 a	12 a	0.55 a	0.59 a
	Subsoil Manure	8.3 a	48 a	53 a	4.2 b	17 a	13 a	0.51 a	0.60 a
	Topsoil	9.3 a	28 b	100 a	3.1 c	21 a	13 a	0.48 a	0.54 a
21	Subsoil	11.4 a	118 a	130 a	6.7 a	17 b	23 a	1.5 a	0.83 a
	Subsoil Compost Manure	8.9 a	74 a	105 a	5.5 a	17 b	17 a	0.55 a	0.72 a
	Subsoil Manure	11.2 a	91 a	98 a	6.5 a	21 b	20 a	0.55 a	0.80 a
	Topsoil	13.6 a	65 a	125 a	6.0 a	37 a	24 a	0.69 a	1.02 a
42	Subsoil	16.1 ab	279 a	147 b	18.1 a	33 a	70 a	0.99 a	1.70 a
	Subsoil Compost Manure	10.3 b	120 b	78 b	9.8 b	32 a	26 c	0.60 c	0.99 c
	Subsoil Manure	14.7 ab	204 ab	123 b	11.0 b	50 a	37 bc	0.71 b	1.24 b
	Topsoil	24.5 a	132 b	236 a	9.8 b	53 a	50 b	1.06 a	1.77 a
56	Subsoil	23.4 bc	335 a	386 a	30.0 a	65 a	120 a	0.84 ab	2.73 a
	Subsoil Compost Manure	17.9 c	182 a	146 a	15.8 b	58 a	50 c	0.71 b	1.54 b
	Subsoil Manure	33.5 ab	300 a	383 a	24.9 ab	88 a	106 ab	0.89 ab	2.83 a
	Topsoil	39.3 a	205 a	560 a	17.1 b	72 a	74 bc	1.14 a	3.04 a
		-----Four Harvest Mean Statistical Significance-----							
Treatment		**	**	**	**	NS	*	NS	**
Harvest		**	**	**	**	**	**	NS	**
Treatment * Harvest		NS	*	**	NS	NS	NS	NS	**

NS=Not significant at 0.05 level, \* significant at 0.05 level, \*\* significant at 0.01 level for average of all four harvests, ANOVA.

Values with same letter for a given nutrient and day after planting are not significant at the 0.05 level, Student Newman Keuls.

is opposite of mycorrhizal colonization (or they were not affected). Copper uptake and shoot weight were essentially the same in the subsoil conventional, subsoil manure and topsoil treatments and was lowest in the subsoil plus composted manure treatment (Table 1). As previously mentioned, reduced yield might be from some unexpected effect of composted manure such as salt or ammonia injury.

## CONCLUSIONS

Spore counts did not help in predicting potential colonization or past observations of yield affects observed in dry bean grown in these soils. These results may not be entirely surprising since high variability in spore counts have been documented (Smith and Read, 1997).

Topsoil and dairy manure additions produced high mycorrhizal colonization of dry bean roots compared to untreated subsoil and this correlated with increased Zn uptake in these two treatments. Increased colonization in the compost manure treatment did not show increased Zn uptake possibly due to negative effects of composted manure on root and shoot growth and mycorrhizal efficiency. Changes in uptake observed with other nutrients were unrelated or negatively related to mycorrhizal colonization percentages. The increased dry bean yields in the field that correlated with increased organic matter and increased Zn uptake observed by Robbins et al. (1997) would be explained by our observations of increased colonization in manure-treated subsoil.

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